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14. ABSTRACT
Signal transducer and activator of transcription 3 (STAT3) is a transcription factor constitutively active in a large number of tumors, where it works as a central player in the activation of multiple oncogenic pathways and contributes to the proliferative state, the migratory and invasive potential and to the maintenance of the mesenchymal phenotype. STAT3 therefore constitutes a potential prime target for directed cancer therapies. A naturally occurring alternative splicing variant, STAT3β, uses an alternative acceptor site within exon 23 and leads to the production of a truncated isoform, which lacks the C-terminal trans-activation domain (TAD). Depending on context, STAT3β can act as a dominant negative regulator of transcription and promote apoptosis. We have used modified antisense oligonucleotides to specifically induce a shift of expression from the abundant, active STAT3a to the truncated STAT3β isoform. Induction of the endogenous STAT3β leads to decreased cell viability in cell lines with persistent STAT3 tyrosine phosphorylation, compared to full STAT3 knock-down obtained by Forced Splicing-Dependent Nonsense-Mediated Decay (FSD-NMD). Furthermore, comparison of the molecular effects of splicing redirection to STAT3 knock-down reveals a unique STAT3b transcriptional signature, with the downregulation of specific target genes (including LEDGF, PCAF, Cyclin C, PEX1 and STAT1b) distinct from canonical STAT3 genes typically associated to total STAT3 knock-down.
Here we propose to screen a next generation panel of antisense compounds, to reprogram STAT3 splicing in cancer cells in vitro and in vivo, in different genetic and orthotopic mouse models, to establish a working model system to test the approach.

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stat3, alternative splicing, antisense, oligonucleotide, cancer, therapy

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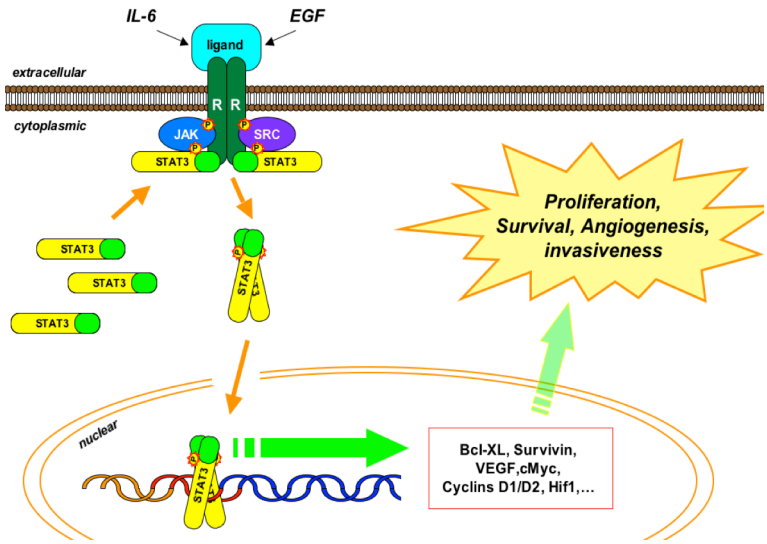
INTRODUCTION

Signal transducer and activator of transcription (STAT) proteins are a family of transcription factors implicated in growth factors and cytokines signalling [1]. In the canonical model of STATs signalling, singles monomers are normally sequestered in the cytoplasm in an inactive form. Their activation is initiated by tyrosine phosphorylation, usually mediated by the binding of cytokines or growth factors to their membrane receptors and/or by intracellular oncogenic tyrosine kinases, such as JAKs and Src. Upon cytoplasmic tyrosine phosphorylation, two STAT monomers dimerize, translocate to the nucleus and bind to specific promoter sequences, thereby regulating gene expression[1]. Under physiological conditions, every phospho-STAT protein has a limited activation period that typically lasts from a few minutes to several hours, while persistent activation of STAT proteins, in particular STAT3 and STAT5, is observed in a wide variety of cancers, including breast cancer[2].

STAT3 is implicated in a vast range of physiological processes including cellular proliferation, differentiation, inflammation and immune response[3-6], but can also act as an oncogene to induce cellular transformation and tumorigenesis[7]. Indeed, persistently tyrosine phosphorylated STAT3 has been reported in nearly 70% of haematological and solid tumors[8]. Because of its wide range of functions, STAT3 is involved in many aspects of carcinogenesis such as proliferation, survival, angiogenesis and metastasis[9,10] and can also contribute to tumor escape from immune surveillance[11,12] and to the establishment of tumor drug resistance[13,14]. Consistently, persistent activation of Stat3, has been reported in human breast carcinoma cell lines but not in mammary epithelial cell lines derived from non-malignant tissues[15,16].

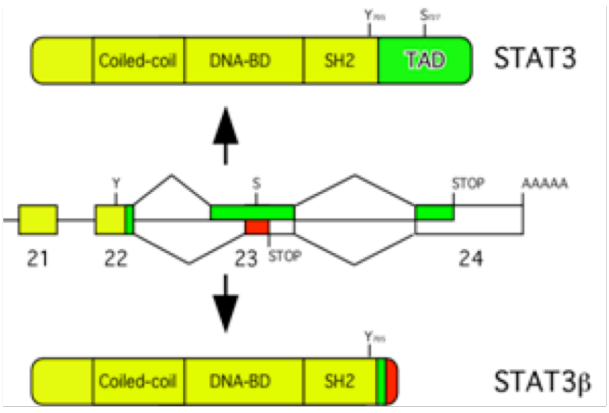
STAT3's pivotal position at the convergence of many oncogenic tyrosin-kinase signalling pathways, makes it a particularly suitable molecular target for cancer therapy,



1. Stat3 signaling. Upon cytokine or growth factor receptor binding, Cyoplasmic Stat3 gets recruited to the plasma membrane and activated via tyrosine phosphorylation by Receptor Tyrosine Kinases (RTKs, such as PDGFR or EGFR), or by non-RTKs (such as Src and JAK). it dimerizes and is translocated to the nucleus where it binds DNA to activate its target genes. Stat3 is tightly regulated by phosphatases, SOCS (suppressor of cytokine signaling) and PIAS (protein inhibitor of activated Stats) and participates into a very complex network of signaling interactions that includes other STAT proteins, additional transcription factors and regulators (not shown).

especially considering that tumor cells tend to become dependent on persistent STAT3 signalling and are more sensitive to its inhibition than normal cells[8,17]. Indeed, direct inhibition of STAT3 activities by multiple means, such as overexpression of dominant negative isoforms[18], antisense oligonucleotides[19], RNAi[20,21] or small drug inhibitors[22] results in growth inhibition and induces apoptosis in breast cancer and other model systems[23]. The direct implication of Stat3 as a promoter of breast tumor growth and progression is further supported by the observation that breast tumor sample show increased Stat3 activity compared to matched non neoplastic tissues, but decreased in samples from patients with a complete pathological response to doxorubicin/docetaxel treatment compared to partial responders[24].

Inihibition of STAT3 activity therefore represents a promising approach in the treatment of breast cancer.

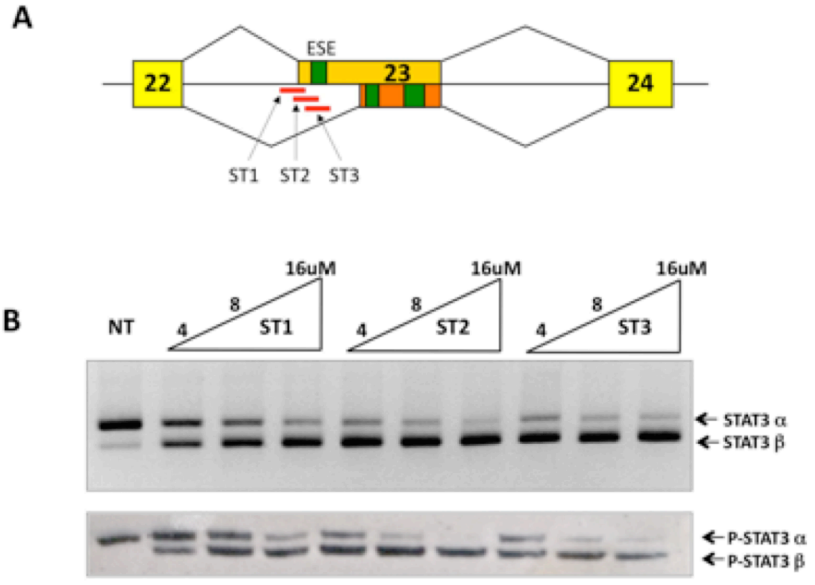


2. STAT3 exon 23 alternative splicing. Alternative acceptor site usage in exon 23 generates both the full length STAT3 transcriptional activator and a dominant negative variant (STAT3 beta) that lacks the transactivation domain (TAD).

A naturally occurring alternative splicing variant, STAT3-beta, uses an alternative acceptor site within exon 23 (Figure 2) and leads to the production of a truncated isoform lacking the C-terminal transactivation domain (TAD). As a consequence, STAT3-beta can still heterodimerize with the full-length STAT3 (STAT3-alpha) and bind to DNA, but it cannot transactivate gene expression, thus effectively blocking STAT3 signalling. Indeed, STAT3-beta can act as a dominant negative regulator of transcription, and its overexpression leads to a significant inhibition of tumor growth and increased apoptosis, both *in vitro* and *in vivo*.

Redirection of STAT3 alternative splicing therefore presents an attractive way to eliminate the tumorigenic STAT3 alpha variant while simultaneously inducing the generation of the STAT3beta variant, which possesses marked anti-tumoral properties.

In the previous CDMRP-BCRP-funded study (BC074961, Modulation of Stat3 Alternative Splicing in Breast Cancer), we induced the STAT3beta isoform using an antisense approach, where modified oligonucleotides were very specifically targeted to splicing regulatory elements to either inhibit or promote the usage of splicing sites[25]. Use of



3. Modulation of Stat3 splicing(A) Schematic localization of the morpholino oligonucleotides relative to exon 23 a 3' splice site and putative mapped ESEs (shown in green). **(B**) (Top) RT-PCR and (Bottom) Western Blot analysis of STAT3 a/b levels in MDA-MB-435s cells treated with increasing concentration of ST1, ST2 and ST3 morpholino oligonucleotides for 4 days.

antisense compounds is a well-established approach for gene expression regulation[26], and normally relies on the destabilization of the target mRNAs, either by triggering RNase H degradation or by taking advantage of the RNAi mechanism. Redirection of splicing differs from the standard antisense-based approach in that the objective is not degradation of the target RNA. On the contrary, triggering of RNase H must be avoided in order to preserve the integrity of the re-directed mRNA, and therefore different chemical characteristics must be present in the antisense compounds.

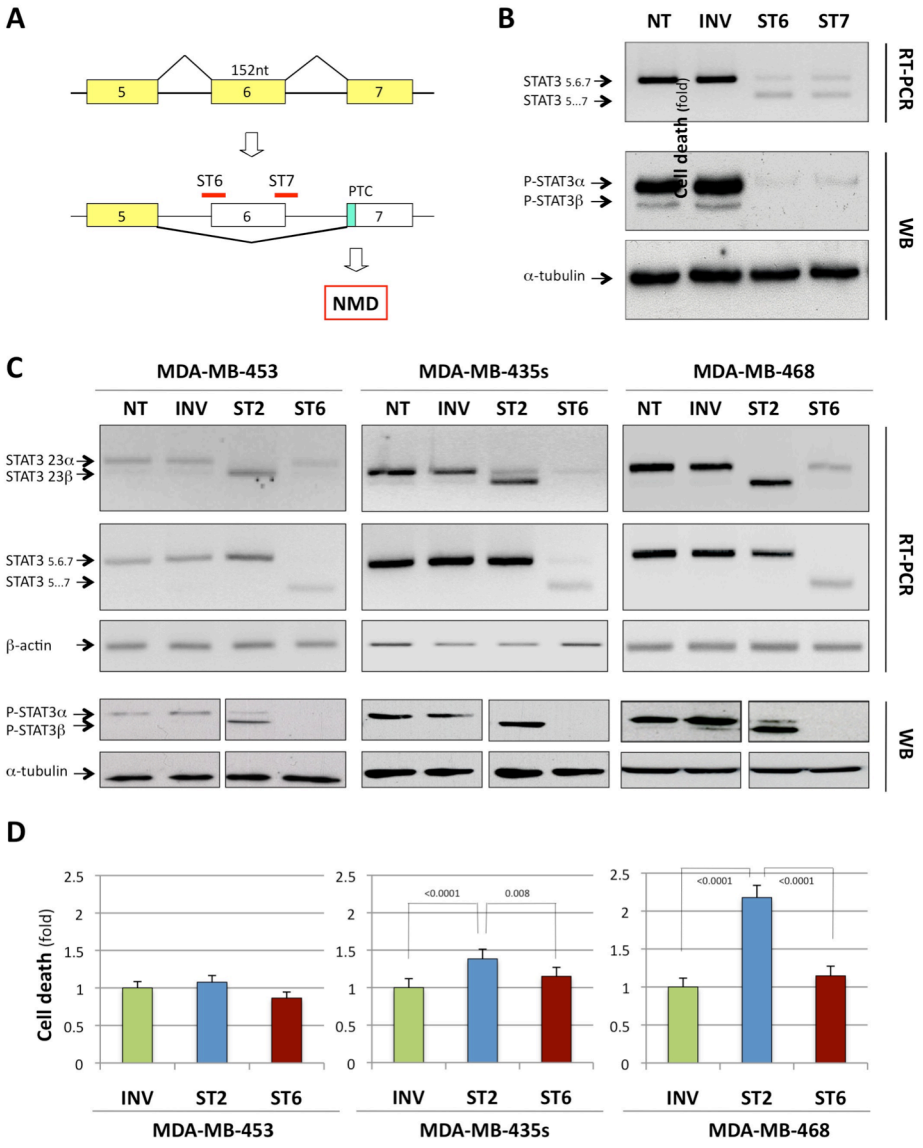
Morpholino phosphorodiamidate oligonucleotides (morpholinos) are DNA oligonucleotide analogues in which the phosphodiester bond is replaced by phosphorodiamidate linkage and the ribose is replaced by a morpholino moiety. Because of its noncharged backbone, morpholinos form very stable duplexes with single-stranded RNA targets and are highly resistant to nucleases and proteases. We had previously developed chimeric compounds that are coupling to the antisense moiety of a peptide (RS-rich) that mimics the action of the "RS domain", the 'activation domain' of splicing factors[27]. In addition, Arginine-rich peptides share structural similarities to translocating peptides (like TAT) that are able to mediate free-uptake of bound cargo into cells. In addition, we have also used a formulation of the compounds (vivo-morpholino) where a cationic dendrimer is coupled to the oligonucleotide instead of the peptide. This variant was originally developed by Gene-tool for *in vivo* treatments.

We thus used splicing redirection compounds to induce a switch from the alpha to the STAT3beta isoform (Figure 3).

To be able to compare the effect of the beta switch to those of a STAT3 knock-down, we adapted the splicing redirection approach to cause a total knock-down by inducing an early splicing shift of a constitutive exon, which leads to a frameshift, a Premature Termination

Codon (PTC) and eventually to degradation of the mRNA by nonsense-mediated decay (NMD). The resulting fsd-NMD (forced splice-dependent NMD) was used to completely knock-down Stat3 as a control (Figure 4 a,b). We then started to assess the biological effect of the switch in breast cancer cell lines that have different extent of Stat3 dependence. The activity was measured using a number of cell-based biological assays.

The alpha-to-beta switch induces cell death in triple-negative Stat3-dependent cell lines like MDA-MB-435s or MDA-MB-468, but not in cell lines such as MDA-453, that do not show persistent activation of Stat3 (figure 4c,d). In side-by-side experiments, however, full knock-down of Stat3 was not as effective as the splicing switch in promoting cell death, suggesting that Stat3beta may induce cell death



4. Effect of STAT3 alternative splicing modulation *in vitro* (**A**) Schematic representation of the strategy used to knock-down STAT3 by Forced Splice-dependent Nonsense-Mediated Decay (FSD-NMD). Morpholino oligonucleotides ST6 and ST7 cause skipping of exon 6 leading to a frameshift and a premature termination codon (PTC), ultimately causing RNA degradation following nonsense mediated decay (NMD).
(**B**) RT-PCR and Western Blot analysis of STAT3 α/β levels in MDA-MB-435s cells treated with 16μM of ST6, ST7 or INV for 4 days. α-tubulin was used as loading control.
(**C**) RT-PCR and Western Blot analysis of STAT3 α/β levels in MDA-MB-453, MDA-MB-435s and MDA-MB-468 cells treated with 16μM of INV, ST2 or ST6 oligos for 4 days. β-actin was used as housekeeping gene for the RT-PCR and α-tubulin was used as loading control for Western blot. NT, untreated.
(**D**) Cell death quantification (represented as fold change relative to control treatment) determined by trypan blue exclusion for MDA-MB-453, MDA-MB-435s and MDA-MB-468 cells treated with 16μM of INV (green), ST2 (blue) or ST6 (red) oligonucleotides for 4 days. Data represent average of ≥ three independent experiments (each experiment done in triplicates). Indicated p-values were calculated by the stratified chi-square test, bars represent standard error of the rate (SE).

by a mechanism different than a straight dominant negative effect.

Indeed, we found that the alpha-to-beta switch activates a specific transcription program that includes downregulation of survival factors, including LEDGF, PCAF, Cyclin C and Stat1beta. Next, we tested whether the splicing redirection compounds also possess anti-tumor activity *in vivo*. The beta-switching compound ST2 and the knock-down compound ST6 were injected intratumorally in athymic mice carrying 435s-derived xenograft tumors [25]. The switch to beta was associated to a full regression of the tumors providing the first evidence that modulation of a single splicing event can have anti-tumoral properties. On the other hand, knock-down of total STAT3 didn't have a significant effect on tumor growth even if the treatment was very effective at the molecular level [25].

BODY

Role of Stat3beta downstream effectors [Cartegni Lab]

Our previous experiments showed that reduction of STAT3a and concomitant induction of STAT3b by alternative splicing modulation can decrease cancer cell viability more efficiently than the knockdown of both STAT3 isoforms. Therefore we set out to determine which STAT3 target genes may be involved in this process.

We thus performed a screen using cDNAs from MDA-MB-435s cells treated for 4 days with ST2 (STAT3b induction), ST6 (STAT3 K.O.) or INV (control) compounds. Expression levels were analyzed by real-time PCR, using the microarray platform U133A 2.0 (Affymetrix) [25]. Whereas knocking-down both STAT3 isoforms by FSD-NMD using the ST6 compound resulted in a somewhat modest but consistent downregulation of most canonical targets analyzed (and a robust downregulation of STAT3 itself, as expected), STAT3b induction by ST2 appears to have activated a STAT3b-specific transcriptional signature [25], indicative of the existence of different classes of target genes in terms of how they respond to STAT3a/STAT3b regulation.

Out of the panel of genes with statistically significant variation of expression levels in the microarray assay, in addition to the previously described STAT3-target interleukin 8 (IL8), five other genes were confirmed to be down-regulated both at the RNA and protein level by the switch to STAT3β, but not by the knockdown: 1.) **Lens Epithelium-Derived Growth Factor** (LEDGF/PSIP1, a chromatin binding protein and transcriptional co-activator; a pro-survival and growth factor)[28]; 2.) **peroxisomal biogenesis factor 1** (PEX1, part of the AAA ATPase

subfamily, required for peroxisomal import)[29]; 3.) **CyclinC** (CCNC, controls transcription by associating with Cdk8 and modulating RNA pol II activity and regulates G0/G1 transition) [30]; 4.) **p300/CBP-associated factor** (PCAF, histone acetyltransferase and transcriptional co-activator, promotes growth, invasion and drug resistance)[31]; and 5.) **STAT1** (proliferative antagonist isoform of tumor suppressor STAT1)[32]. In the case of STAT1, the effect is indirect or post-transcriptional, as only the alternative splicing variant STAT1b, but not the full-length variant STAT1a is affected). Notably, the microarray analysis confirmed that none of the canonical STAT3 targets, except for IL8, were strongly down-regulated by the STAT3β induction. LEDGF, PEX1, Cyclin C and STAT1b were also specifically down-regulated by the switch from STAT3α to STAT3β in MDA-MB-468 cells.

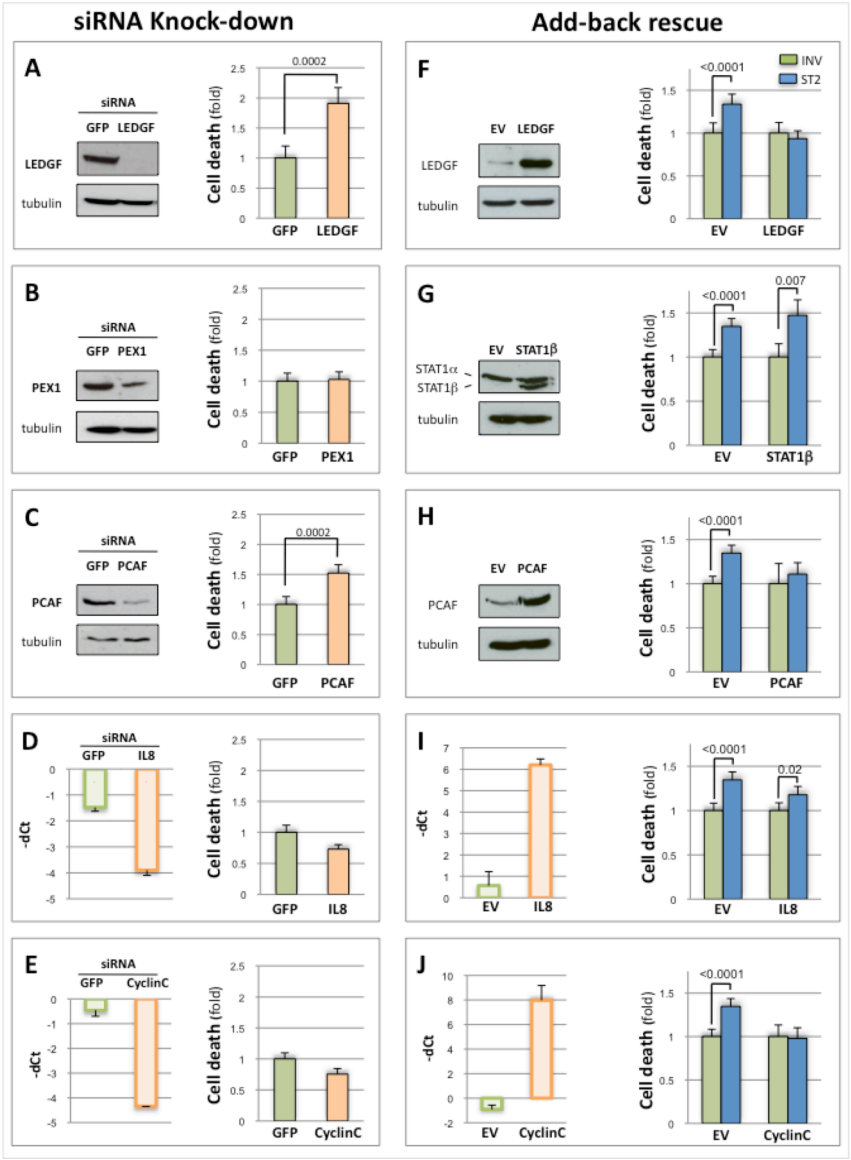


Figure 5. Knock-down and overexpression of STAT3β target genes. (**A-E**) (Left) MDA-MB-435s were treated for 72 hours with siRNA against GFP or: LEDGF (A), PEX1 (B), PCAF (C), IL8 (D) or CyclinC (E). Effective knock-down was verified by WB using specific antibodies (A-C, with α-Tubulin as control) or by qPCR analysis (D-E), displayed as -dC(t) values after normalization to HPRT. (Right) Cell death quantification determined by trypan blue exclusion for MDA-MB-435s treated as described. Data are displayed as fold change of treated samples (orange) relative to control treatment (green) and represent average of three independent experiments (each in triplicates). P-values were calculated by the stratified chi-square test, bars represent standard error of the rate (SE). **(F)** (Left) Lysates from MDA-MB-435s transiently over-expressing LEDGF or transfected with empty vector (EV) were immunoblotted using antibody to LEDGF. (Right) The transfected cells were concurrently treated with 16uM of INV or ST2 morpholinos for 4 days, and cell death was quantified as above. **(G-J)** (Left) Stable MDA-MB-435s clones over-expressing STAT1β (G), PCAF (H), IL8 (I), CyclinC (J) or selected for the empty vector (EV) were treated with 16uM of INV or ST2 morpholinos for 4 days. Over-expression levels were determined by WB using specific antibodies (G-H, with α-tubulin used as loading control) or by qPCR analysis (I-J), displayed as -dC(t) values after normalization to HPRT. (Right) Cell death quantified as above.

Knockdown and rescue of STAT3β targets [Cartegni Lab]. The set of target genes specifically down-regulated when STAT3 splicing is redirected from STAT3α to STAT3β had not previously been associated with STAT3β activity. In order to get a better understanding

of their contribution to the biological effects observed, we took two different approaches. First, we tested whether their individual down-regulation was able to recapitulate the decrease in cell viability observed with the ST2 treatment. MDA-MD-435s cells were treated with siRNA against five target genes (no siRNAs are available to specifically target STAT1b but not STAT1a). Effective knock-down was verified by qPCR and WB, where suitable antibodies were available (Figure 5 A-E) and cell viability was measured. Individual down-regulation of PEX1, IL8 or CyclinC did not lead to an increase in cell death (Figure 5B, D, E). On the other hand, knock-down of LEDGF (Figure 5A) and PCAF (Figure 5C), was associated to a significant decrease in cell viability.

Next we asked whether forced re-expression of the STAT3β target genes could protect from the increase in cell death induced by the α-to-β switch. We thus generated MDA-MB-435s-derived cell lines stably expressing four of the STAT3β target genes (STAT1b, PCAF, IL8, CyclinC). Initially we were unable to generate a stable LEDGF-expressing line, therefore the equivalent experiment (shown) was performed under transient expression conditions. However, additional stable clones are under characterization and once obtained will be also used for successive phases of the proposed experiments.

Cells were concurrently treated in vitro with either ST2 or INV compounds, and cell viability was measured after 4 days. Forced expression of STAT1b did not protect from cell death induced by the splicing switch (Fig. 5G), compared to cells selected for integration of the empty vector. Expression of IL8 (Fig. 5I) showed a reduced but still significant increase in cell death.

Most importantly**, LEDGF, PCAF and Cyclin C** protected cells from the α-to-β shift (Fig. 5F, 5H-J) as treating the overexpressing cells with ST2 had no effect on their viability, unlike what observed with control cells. Taken together these data suggest that the effect of the STAT3β isoform on cell viability is likely mediated by the combined down-regulation of a specific set of target genes rather than exerted through a single major effector, in agreement with STAT3 pleiotropic functions in the pathogenesis of cancer.

In vivo treatment of stat3-dependent tumors [Cartegni lab, de Stanchina lab]

In preliminary experiments, we have tested how STAT3 alternative splicing modulation could affect tumor growth in vivo, using the ST2, ST6 and INV morpholino compounds described earlier, delivered by intratumoral injection. Athymic mice subcutaneously implanted with MDA-MB-435s cells were IT injected twice a week for a total of 3 weeks while monitoring for tumor growth. 13 days after the first injections tumors treated with the switching ST2

morpholino showed regression (Figure 5A-B) while tumors treated with the knock-down ST6 compound grew as well as PBS and INV-treated tumors [25].

RT-PCR for either STAT3 exon 23 (Figure 5C) or STAT3 exon 6 (Figure 5D) confirmed efficacy of both ST2 and ST6 treatments in inducing either STAT3 splicing switch or total STAT3 FSD-NMD knockdown. Tumor sections were analyzed by immunohistochemistry (IHC) with three STAT3 antibodies. Staining for total-STAT3 and P-Y705 (present in both STAT3α and STAT3β) was strongly decreased in ST6-treated tumors (Figure 5F, 5H), confirming that the ST6 compound was effective at substantially knocking down STAT3. On the contrary, the level of both total-STAT3 and P-Y705 in ST2-treated tumors was comparable to control-treated tumors, consistent with the persistent expression of STAT3β and the maintenance of this phosphorylation site (Figure 5F, H). IHC for P-S727 (present only in STAT3α) was negative for both ST2 and ST6 treated tumors, further confirming the efficacy of these treatments at the protein level (Figure 3G). Analysis of tumor sections stained for Hematoxylin and Eosin (H&E, Figure 3I), showed that the ST2-induced splicing switch is the only treatment associated with a significant effect on tumor morphology. Of particular interest is that while STAT3 knock-down was effective at the

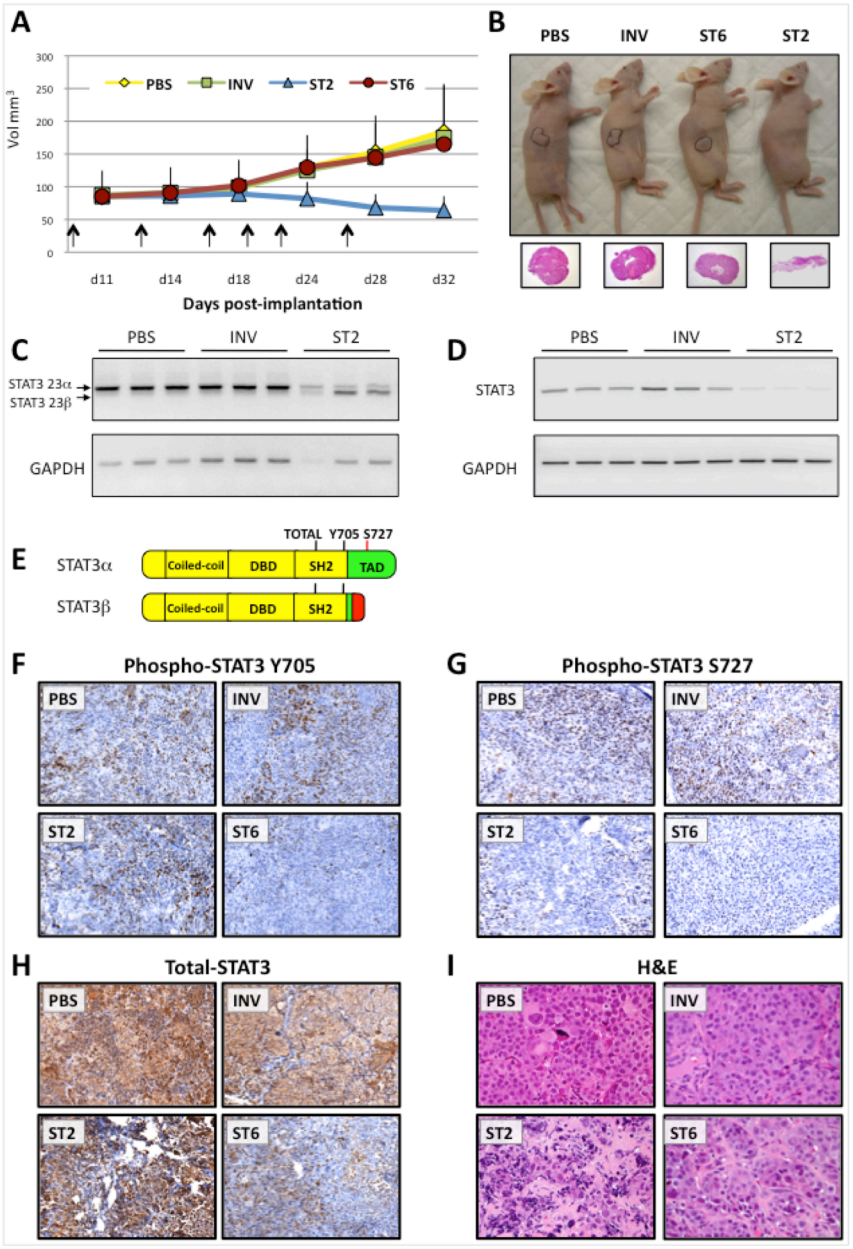


Figure 5. Effect of STAT3 alternative splicing modulation *in vivo*. (**A**) Following subcutaneous injection of $10x10^6$ MDA-MB-435s cells, tumors of ~100 mm^3 were treated intratumorally with 0.12mg of vivo-Morpholino (PBS, INV, ST2 and ST6) twice a week for 3 weeks (arrows) and tumor volumes were measured. **B**) Representative image of mice in each treatment group at time of sacrifice, with H&E staining of tumor sections underneath. (**C, D**) RT-PCR analysis of STAT3 exon 23 and exon 6 levels (respectively) in three tumors for each treatment group. GAPDH was used as control. **(E)** Epitopes' location of STAT3 antibodies used for the IHC staining. **(F, G and H)** Representative images of tumor sections from the 4 different treatment groups following IHC using specific antibodies against P-Y705, P-S727 and total STAT3, respectively. **(I)** Representative images of H&E stained tumor sections from the 4 different treatment groups.

molecular level, it did not have any notable effects on tumor morphology and growth compared to controls (Figure 3I).

Expression of LEDGF, Cyclin C, STAT1b and PCAF and IL8 is also inhibited in MDA-MB-435s-derived tumors in mice upon STAT3b activation, like previously described for the MDA-MB-435s cell lines. To assess whether their suppressive role in STAT3b-induced cell death also applies to in vivo tumor growth and progression when overexpressed, we started set up an in vivo system using the stable cell lines described above.

In experiments carried out in the de Stanchina lab, new baselines for tumor growth were derived in 8-10 weeks old athymic female mice, which were subcutaneously inoculated with 10X106 MDA-MB-435s cells expressing STAT1b and PCAF. In addition, MDA-MB-435s-derived CyclinC and LEDGF cell lines are being currently re-generated from novel individual clones, as the expression in the previous clones resulted unstable. Next, a set of experiments using MDA-MB-435s cells overexpressing PCAF, STAT1b and control MDA-MB-435s, divided in 3 treatment group each (mock, INV and ST2 for splicing re-direction, 5 mice per group) have been started, with Vivo-Morpholino administered intratumorally at a final concentration of 0.12mg/30ul, twice a week. Tumor-bearing mice are assessed for weight loss and tumor volume twice weekly. Tumor volumes were determined by caliper. Tumor samples were harvested, to be analyzed by immunohistochemistry and at the RNA and protein levels. Unfortunately there was an issue with tumor up-take, so the results of these experiments are not conclusive and will need to be repeated for statistical significance. However, our initial indications suggest that in the presence of overexpressed PCAF (and to a lesser extent STA1b) the treatment with the splice-switching ST2 vivo-morpholino compound leads to a reduction in tumor growth, although such reduction does not appear to be as effective as when PCAF is not over-expressed, in which case a full tumor regression is observed. This is important, because (if confirmed) it suggests that also in this xenograft in vivo system, PCAF plays a key role in mediating Stat3beta anti-tumoral activity.

As well as repeating and completing the experiment delineated above, we have also designed FSD-NMD morpholino compounds to knock-down endogenous PCAF expression, and to induce endogenous expression to activate STAT1b. These have now been synthesized to be tested for activity in cell lines [Cartegni lab], Active compounds will then be used by themselves and in combination to test their anti-tumoral activity, to show that the anti-tumorigenic activity of the ST2 compounds is mediated by PCAF and/or STAT1b [de Stanchina lab].

In parallel, we have designed and synthesized morpholino compounds specific to either the human (ST2h) or mouse STAT3 exon 23 sequence (ST2m), which induce activation of

STAT3b in either species but not in both. These have now been cross-tested in human and mouse cell lines to confirm that they can effectively and specifically activate STAT3beta in a species-specific way [Cartegni lab]. Similarly to previous experiments, the comparison in vivo using the MDA-Mb-435s model to compare targeting the tumor proper exclusively (ST2h) vs. targeting the tumor environment exclusively (ST2m) vs. targeting both (ST2) are ready to be undertaken in the de Stanchina lab.

Finally, next-generation compounds, based on alternative chemistry (2'MOE) and alternative delivery system (peptide-based) have been designed and synthesized and initial testing are undergoing in the Cartegni lab. Similarly, initial optimization procedures to establish a second, complementary model of aggressive breast cancer using MDA-MB-231 subpopulation LM2-4175[33] cells subcutaneously (as above), systemically (via injection into the left ventricle) or orthotopically into axillary mammary fat pads of 6-week old female athymic nude mice are underway in the de Stanchina lab. This cell line expresses luciferase, which allows for accurate quantification of tumor growth, and induces lung metastases, which will allow us to test for compound efficacy in metastatic conditions. For mammary-fat-pad tumor assays, 3 million cells resuspended in a 50:50 solution of PBS and Matrigel are directly injected into the mammary fat pad corresponding to gland #3. For cardiac injections, 1 million cells resuspended in 100 ul PBS are injected into the left ventricle of anesthesized mice. Tumor progression is visualized by weekly imaging. To do so, mice will be injected with D-luciferin (Xenogen) at 50 mg kg and Photonic emission captured with the In Vivo Imaging System (IVIS, Xenogen). Tumor bioluminescence is then quantified by integrating the photonic flux (photons per second) through a region encircling each tumor, as determined by the LIVING IMAGES software package (Xenogen).

Calibration experiments for imaging have been performed and usage of this model with the ST2 compound will begin in the next funding period.

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KEY RESEARCH ACCOMPLISHMENTS

1. **Generation of improved stable MDA-MB-435s cell lines overexpressing LEDGF and CyclinC**
2. **Effect on tumor growth of overexpression of PCAF or STAT1b**
3. **Effect of STAT3 alpha-beta induction on growth of tumors overexpressing PCAF or STAT1b**
4. **Design and testing of morpholino compounds targeted to PCAF (FSD-NMD) and STAT1b (splice-switching)**
5. **Design and testing of morpholino compounds specifically targeted to mouse or human STAT3 exon 23**
6. **Design an testing of 2'MOE compounds**
7. **Establishment and calibration of the MDA-MB-231 (LM2-4175) model system**

REPORTABLE OUTCOMES

1. **Lee Spraggon and Luca Cartegni;** Antisense Modulation of RNA Processing as a Therapeutic Approach in Cancer Therapy, *Drug Discovery Today: Therapeutic Strategies* (2013) http://dx.doi.org/10.1016/j.ddstr.2013.06.002

CONCLUSIONS

STAT3 is an oncogene transcription factor whose constitutive activation in a large majority of tumors, including breast cancer, is thought to contribute to multiple aspects of the tumorigenesis process. Its main alternative splicing isoform, STAT3b, can act as a dominant negative factor that, when overexpressed in cancer cells, is able to inhibit cell growth in vitro and in vivo. In addition to its dominant negative functions. STAT3b is also in direct transcriptional control of some specific pathways, with antitumorigenic properties, which are independent of the full length STAT3 activities. This Sta3b-specific targets include PCAF, LEDGF, IL8, Cyclin C and STAT1b.

We have previously shown that the modulation of STAT3/STAT3b relative expression levels via splicing re-direction compounds, can lead to a potent anti-tumorigenic approach trough the modulation of the above mentioned STAT3b specific set of target genes.

The first funding period of this project has been instrumental in the setup of numerous experimental systems, with the multiple targets described in the specific aims of the project. In particular, a lot of the early work in the Cartegni lab has been focused on the exploration of the specific role of the a few STAT3b targets (PCAF, LEDGF, IL8, STAT1b) with the determination of their causal involvement in anti-tumorigenic activities.

In addition, the design, synthesis and initial testing of new compounds to regulate the above mentioned targets and to develop next generation, more active STAT3 splicing redirection compounds, or compounds that interrogate the role of STAT3beta in the tumor proper or in the tumor environment has been carried out in the Cartegni lab.

At the same time the de Stanchina lab has proceeded in the establishment of multiple novel in vivo systems, including those aimed at assessing the role of the above mentioned STAt3b targets in vivo, those aimed at testing the role of Stat3beta in the tumor microenvironment and those directed at the establishment of a second, aggressive breast cancer system which incorporates a luciferase readout, This will also provide the possibility to assess the effectiveness of all the above compounds (first and second generation) by themselves or in combination with traditional chemotherapy in a metastatic model of breast cancer.

A major hurdle, which has been associated with the interruption of the research project at the end of the first funding period, has been the transfer of the laboratory of Dr. Cartegni's from Memorial Sloan-Kettering Cancer Center, New York, to the Susan Lehman Cullman

Laboratory for Cancer Research, Ernest Mario School of Pharmacy, Rutgers University, New Jersey.

The transfer initiated at the end of September 2013 has taken significantly more time than expected and the new laboratory has became fully operative only in February 2014. In addition to the 4 months ‘down-time’, additional delays compared to the original timeline are expected because of the need to train and bring up to speed the new personnel tha have been hire for the task.

Importantly, no DOD funds from this grant have been used, at MSK or at Rutgers, since the move has started in September 2013 in order to wait for the laboratory to become fully operative and the fund situation to be clarified. No changes occurred within the lab of Dr. de Stanchina (MSKCC), so nothing is expected to change regarding that part of the project, although experiments have been suspended for the delay period in her laboratory as well.

Lehman Cullman Laboratory for Cancer Research at Rutgers university is a prestigious institution that will fully provide all the needed equipment, support and expertize needed to carry out the project as anticipated.

The only significant difference anticipated is that we will be asking for a no-cost six months extension at the end of the final funding period to compensate the delay due to the move of Dr. Cartegni’s lab.

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APPENDICES	none

SUPPORTING DATA	none